FUCOSYLATION INHIBITOR DEVELOPMENT FOR PRODUCING AFUCOSYLATED ANTIBODIES

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Fucosylation is an important quality attribute for therapeutic antibodies. Afucosylated antibodies exhibit higher therapeutic efficacies than their fucosylated counterparts through the antibody-dependent cellular cytotoxicity (ADCC) mechanism. In ADCC, antibodies with less fucosylation bind more tightly to the leukocyte receptors of the FcγR family, leading to enhanced cellular cytotoxicity. Since higher potency is beneficial in reducing dose or duration of the treatment, afucosylation antibodies have attracted a great deal of interests in biotherapeutics development. Different cell line engineering approaches have been used to generate afucosylated antibodies in CHO cell lines. Knockout (e.g., α-1,6-fucosyltransferase (FUT8)) or overexpression (e.g., acetyl-glucosaminyltransferase III, GDP-mannose 6-dehydrogenase, etc.) of key enzymes involved with fucosylation was effective in generating afucosylated antibodies in multiple applications. However, establishing cell lines with gene knockout or overexpression is often a lengthy process.

In this study, we synthesized novel small molecules to inhibit the enzyme in the GDP-fucose synthesis pathway. With the presence of inhibitors, GDP-fucose synthesis is blocked and it becomes insufficient for antibody fucosylation. The inhibition studies were conducted in fed-batch cultures and the chemicals were added at different concentrations. Our data showed that inhibitor addition increased antibody afucosylation levels in a dose-dependent manner and had no significant impact on other protein quality attributes. We demonstrated that modulating antibody afucosylation levels can be achieved by this relatively easy yet robust approach. Those novel small molecules can be used to generate antibodies with different afucosylation levels as required for discovery studies in R&D, analytical method development, and other applications.